Quality characterization of the olive oil from var. Tosca 07® grown in a commercial high density orchard

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ABSTRACT

High density olive orchard is a new planting system that requires cultivars with low vegetative vigor, such as Arbequina and Arbosana varieties. Different cultivars provide different performances in such orchards. This research was performed in order to determine the behavior of the new olive variety Tosca 07® in a commercial, high density orchard. The quality of Tosca 07® olive oils in three different maturity degrees during two crops seasons by physico-chemical and nutritional characterization were compared with Arbequina olive oils obtained from trees grown in the same conditions. Tosca 07® is a very interesting olive variety for high density orchards. Because of their early ripening, they would be suitable for early harvesting, and this could be interesting to avoid the cold temperatures, frost, etc. Tosca 07® olive oils also demonstrated a very convenient chemical composition in comparison with Arbequina olive oils, especially for the high content of antioxidant compounds (α– tocopherol and pigments) present within the oils.

INTRODUCTION

Olive oil- the olive juice- is a very interesting food with excellent sensorial and nutritional properties. The fatty acid composition- very rich in the monounsaturated oleic acid- and the antioxidant compounds (phenols and alpha- tocopherol) give a product very important in Mediterranean diet. The olive oil chemical composition depends of many factors as genetics, extraction process, agronomic factors as production area, climatic conditions and also production system (traditional, medium density or high density orchard). Medium density and high density orchards are characterized by irrigation and high density of trees. These systems are used last years in main producers countries. For these orchards special cultivars are needed adapted to the high density (small size of the trees) and mechanical harvesting. Mostly Arbequina cultivar is used in medium density and high density systems. Other varieties as Arbosana, Koroneiki, Sikitita, etc. are used (1,2). Along ripening there are many modifications in olives and this changes will affect the olive oil quality and chemical composition (fatty acids, phenol content, tocopherol content etc.).

Tosca 07® is an olive variety obtained from the genetic breeding program started by Attilio Sonnoli in 1962 year. In fact, Tosca 07® started from the precursor Italian variety, Urano (3, 4, 5, 6, 7). These olive trees are adequate for medium density and high density orchards because of their compact size and low vigor. There are just a few studies about olive oil quality of this variety (8), but just in only a maturity degree and also with a few analytical parameters. The olive oil quality of the different varieties is described by characterization of their compounds (9,10,11).

The aim of this work was to describe in an initial study the quality of Tosca 07® olive oils obtained from a commercial high density orchard (during two crop seasons and in three different maturity degrees) by physico-chemical and nutritional characterization, compared with Arbequina olive oils grown in the same conditions.

EXPERIMENTAL PROCEDURES

Olive fruit sampling

The trial was carried out during the crop seasons 2011 and 2012 in a commercial, irrigated orchard (*Olea europaea L.* cv. Arbequina and Tosca 07®) located in Zaragoza (Spain) planted in 2009 with a distance of 1.5 m on the trees and 4.5 m between rows (1600 trees/ha). Samples were handpicked weekly three times on 19th October, 26th October, and 2th November. Each sample consisted of 5000 olives from different trees in perfect sanitary conditions. Temperatures and rainfall were registered during all the sampling period.

Ripeness index and morphologic parameters of the olive fruits

The olive ripeness index (RI) was determined using the method developed by the Agronomic Station of Jaén (12) based on the evaluation of the pigmentation of olive skin and pulp.

One hundred olives were also randomly selected in order to calculate their average weight (g) and diameters ecuatorial and polar (mm).

Oil content

An olive paste was prepared by milling. After drying at 105ºC, the extraction of oil content was performed by Soxtec™ 2055 using petroleum ether as a solvent at 115ºC.

Oil extraction process

Oil extraction was done using an Abencor laboratory equipment, using the method described by Martínez et al. (13). The olives were cleaned and crushed with a mill, and the paste was malaxated at 26º C ±1ºC for 30 min and then centrifuged at 3500 rpm for 1 min. After filtration, olive oil samples were stored at -18º C in darkness using amber glass bottles with nitrogen in the headspace prior to analysis.

Analytical determinations

Determinations of the physicochemical quality parameters (free acidity, peroxide value, and UV absorption characteristics, K_{270} and K_{232}) were performed following the official methods described in Regulation EEC/2568/91 of the Commission of the European Union (14).

Determination of fatty acids. The fatty acid profile of the olive oils was determined by GC using a modified fatty acid methyl esters (FAMEs) method, as described by Frega and Bocci (15). The FAMEs were prepared by shaking a solution of each olive oil in n-hexane and 2N methanolic potassium hydroxide. Chromatographic analyses were done using a GC instrument equipped with a flame ionization detector and a split/splitless injector. The experimental conditions used were: DB- 225 column. The injector and detector temperatures were 250 ºC. The oven temperature was programmed from 190 ºC (1 min) to 210 at 4 ºC/min and maintained for 5 min, then heated to 215 at 3 ºC/min, and, finally, an isotherm was used for 18 min. Nitrogen was used as the carrier gas. Commercial mixtures of fatty acid methyl esters were used as reference data for the relative retention times. Results were expressed in percentage of the total fatty acids.

α-tocopherol determination. A solution of oil in hexane was analyzed by HPLC with a Zorbax SB-C18 phase-reverse column, eluted with acetonitrile/water (99:1 v/v) at a flow rate of 1 mL/min. The injection volume was 20 µl. A photodiode matrix detector (G1315B, series 1100) was used. Chromatograms were registered at 295 nm. Results were expressed as mg α-tocopherol/Kg oil.

Total phenol content. The extraction of the total phenols from the olive oil, was done following the method described by Favati et al. (16). The phenols were extracted by Solid Phase Extraction (SPE) using Isolute C18 cartridges (6 ml/1g solid phase). The extract was dried at 40ºC in a rotary evaporator, and the residue was dissolved in 5 ml methanol. For the colorimetric determination of total phenols, 2.5 ml of extract was mixed with 1.25 ml of Folin-Ciocalteau reagent, and after 3 min, 2.5 ml of sodium carbonate was added. The absorption of the solution was measured at 725 nm. Results were expressed as mg gallic acid/ Kg oil.

Individual phenols. Phenolic compounds were extracted from olive oil following the method described by Gutfinger (17). A HPLC was used for the sample analysis. The column was a Zorbax SB-C18. The eluents were 0.2% aqueous acetic acid (pH 3.1) and methanol, the flow rate was 1.5 ml/min, and the injection volume was 20 µl. The total run time was 60 min. The initial composition was 95% aqueous acetic acid and 5% methanol, and the gradient changed as follows: The concentration of methanol was maintained for 2 min, then it was increased to 25% in 8 min, and, finally, the methanol percentage was increased to 40, 50, and 100% for 10 min at each of the percentages. Initial conditions were reached after a total of 15 min. Chromatograms were obtained at 280 nm and 339 nm. Phenolic compounds were identified on the basis of their retention times compared to those of the standard compounds. The quantitative determination was performed using standards. The results were expressed as mg/Kg oil.

Oxidative stability. A Rancimat 743 apparatus was used to obtain the oxidation induction time (hours). An oil sample of 3 g was warmed to 120º C with 20 l/h air flow. The induction time is the time to reach the break point of this curve.

Determination of chlorophyll and carotenoid compounds. Chlorophyll and carotenoid were determined from the absorption spectra of the olive oil for each sample (7.5 g) dissolved in cyclohexane (25 ml) following the method of Minguez-Mosquera et al. (18). The maximum absorption is related to the chlorophyll fraction at 670 nm and to the carotenoid fraction at 470 nm. The values of the coefficients of specific extinction applied were E0 = 613 for pheophytin (major component in the chlorophyll fraction) and E0 = 2000 for lutein (major component in the carotenoid fraction). The concentrations of chlorophyll and carotenoids were expressed as mg pheophytin and lutein/ Kg oil, respectively.

Color measurement. The CIELAB color space (CIE) was studied with an AvaSpec-1024 spectrophotometer after the spectra were obtained. Illuminant D65 was chosen, along with observer CIE64. The following color coordinates were determined: lightness (L*), redness (a*, red/green), and yellowness (b*, yellow/blue).

Bitterness index. Bitterness (K_{225}) was determined by SPE of bitter compounds, using Isolute C18 cartridges (6 ml/500 mg solid phase). The oil was dissolved in *n*-hexane, the cartridge was conditioned by eluting with methanol and *n*-hexane, and the oil was applied to the SPE column. The column was washed with hexane, which was run through the cartridge and discarded. The bitter compounds were eluted with methanol/water (50:50). The absorbance of the methanolic extract was measured at 225 nm. The results were expressed as the absorbance of 1 g oil/100ml.

Statistical analysis

Statistical analysis was performed using Statgraphics Plus 5.1. Results were expressed as mean ± standard deviation of three experiments and as least squares mean ± 95% confidence interval.

Significant differences between samples were determined by analysis of variance (One way ANOVA) and Multiple Range Test.

RESULTS AND DISCUSSION

The ripeness index of Tosca 07® and Arbequina olives was significantly different at the same dates (Table 1) and was increasing along dates of sampling. Tosca 07® olives had a higher value than Arbequina olives, reaching a 4.4 index in the last sampling (2.7 for Arbequina) in 2011 harvesting and 3.8 index (2.4 for Arbequina) in 2012 harvesting. The temperatures and rainfall for the two crop seasons are shown in Figure 1 and Figure 2. The reasons for a higher maturity index in 2011 crop season may be is due to higher temperatures. Also in 2012 some rainfall were registered so the sun incidence would be smaller and that´s right maturity was slower. For this reason, Tosca 07® olives will be suitable for early harvesting, and this could be interesting to avoid the cold temperatures, frost, etc. As a consequence, also, the weights of the fruits, as well as the diameters, were higher for Tosca 07® olives at the same dates (6). Most probably, the ripening is still not finished in the Arbequina olives at the end of the sampling process. In spite of this, the oil content in the olive paste was higher in Arbequina in comparison with Tosca 07®**.**

The olive oil-regulated parameters (acidity, peroxide value, K_{232} and K_{270}) (Table 1) in all of the cases was within the limits established by European regulations for extra virgin olive oil (14). Along ripening free acidity was increasing in all the samples slightly. Similar results were described in other super-intensive orchards (19). Since acidity is a regulated parameter for commercial classification and for a better quality low acidity is necessary is good to choose the harvesting date more early. Peroxide value and K_{232} and K_{270} were higher for Tosca 07® olive oils.

The fatty acid profile (Table 2) showed some significant differences for both Arbequina and Tosca 07® olive oils. Other previous works used this profile to show the authenticity of the olive oil (20) The fatty acid content in all of the sampling dates and in both of the varieties was between the limits established by regulations for extra virgin olive oil (linolenic $\leq$ 1%, arachidic $\leq$ 0.6%, and gadoleic $\leq$ 0.4%). The main fatty acid in all the samples was oleic acid with more than 63% of the total content This content was higher in Arbequina olive oils. Linoleic acid was higher for Tosca 07® olive oils and increased slowly throughout ripening. On the other hand, linolenic acid was also higher for Tosca 07® olive oils, decreasing along sampling. MUFAS content was significantly higher in Arbequina olive oils, and PUFAS content was significantly higher in olive oil from Tosca 07® variety. In this sense, Tosca 07® olive oil will be very interesting, as PUFAS are essential fatty acids and must be included in the diet.

Phenols are very interesting compounds in olive oil. First of all they have antioxidant properties but also they give to the olive oil some sensorial properties as the bitterness and pungency. Total phenol levels (Figure 3) decreased in general during ripening. In our previous works (21), we found in Arbequina olive oils from high density orchards that phenols increased until they reached a maximum and then decreased. This could be explained because in the previous work, the sampling started at 15 September, and the maximum was reached in October. In this work, we started the sampling at 19 October, probably after this maximum. Differences were observed between the values of the total phenol content obtained from Tosca 07® and Arbequina olive oils. The highest values were obtained from Arbequina olive oils in the 2011 crop season, ranging along ripening from 273 to 252 mg/Kg. In the 2012 crop, the total phenol content was higher for Tosca 07® olive oils. In Tosca 07® olive oils, the total phenol content value was from 120 to 71 mg gallic acid/ Kg in 2011 and 209 to 158 mg gallic acid/ Kg in 2012.

Individual phenols (Table 3) showed some significant differences between the two olive oils. With regard to simple phenols, such as tyrosol, these compounds are more abundant in oils from Tosca 07®olive oil. Other authors described similar results for Sikitita and Picual olive oils in comparison with Arbequina olive oils (22). The same behavior was observed for 3,4-DHPEA-EA, lignans, luteolin, and apigenin. However, vanillic acid, vanillin, coumaric acid, and 3,4-DHPEA-EDA were all substantially higher for Arbequina olive oils (23).. In addition, 3,4 DHPEA-AC and p- HPEA-EDA does not follow homogeneous behavior between varieties and crop seasons. Secoiridoid derivatives play an important role in oil stability; these have already been evaluated and have been shown to extend the shelf life of olive oil. The secoiridoid derivatives of hydroxytyrosol seemed to be the major phenolic fraction in Arbequina olive oils, especially 3,4-DHPEA-EDA. In Tosca 07® olive oils, one of the major phenolic fractions was lignans. In the individual phenols climatic conditions are very important specially freeze damage during harvesting since secoiridoid derivatives decreases due to enzymatic oxidation (24). In this study these damage are not observed and that´s right the early ripening in Tosca 07®olives is very important to avoid the frost.

α-tocopherol content (Figure 4) was higher in Tosca 07® olive oils in both crop seasons and was decreasing along ripening, ranging from 415 to 358 mg/Kg in the 2011 crop season and from 442 to 407 mg/Kg in the 2012 crop season. This content was higher than in other Spanish varieties, and it will be similar to the content of other varieties, such as Arbosana. This must be appreciated because of the antioxidant and nutritional properties of this component. For this reason, a Tosca 07® monovarietal olive oil will be very interesting from a nutritional point of view (25,26,27).

The oxidative stability measured is shown in Figure 5. This value decreased along ripening for Tosca 07® olive oil following the same shape that total phenols For Arbequina olive oils the oxidative stability was similar in each year. During these three picking dates none change was observed in the values of oxidative stability. The values for oxidative stability were lower for Tosca 07® olive oils, probably for the lower content of phenols in the 2011 crop season. Although the total phenol content is higher for Tosca 07® olive oils in the 2012 crop season, oxidative stability was lower, probably due to the higher content of PUFAS**.** Even if phenols and α- tocopherol are antioxidants components may be the contribution of phenol content (specially 3,4-DHPEA-EDA), to the oxidative stability is bigger, and for this reason, Arbequina olive oils are more stable to oxidation.

Pigments are important because of their antioxidant properties but also because they give to the olive oils their characteristic color. The pigment content (Table 4) decreases significantly along ripening in both olive oils. These results agree with the previous research described by Gutierrez et al. (28). The pigment content was, in general, higher in Tosca 07® olive oil in both chlorophyll and carotenoids content. However, at the end of sampling, the pigment content was similar for both olive oils. The concentration of chlorophylls ranged from 8.0to 4.1 mg/Kg in Arbequina olive oils in 2011 (9.9 to 4.2 in 2012) and from 11.2 to 3.4 mg/ Kg in Tosca 07® olive oils in 2011 (12 to 3.4 in 2012). The concentrations of carotenoid pigments also decreased markedly throughout the period, in oils from both Tosca 07® and Arbequina. These results agree with those found from other authors when comparing Arbequina olive oils with Picual and Sikitita olive oils (29). The concentration of carotenoids ranged from 6.8 to 3.6 mg/Kg in Arbequina olive oils in 2011 (6.6 to 3.5 in 2012) and from 9.1 to 4.0 mg/ Kg in Tosca 07® olive oils in 2011 (7.6 to 3.8 in 2012).

In other side color coordinates are describes in Table 4. L* values were smaller in Tosca 07® olive oils because of their higher pigment content. Along ripening L* value was increasing in all the samples with a behaviour opposite to the pigmen content. In addition, the b* values decrease significantly along ripening in all of the olive oils. Also, the a* values were more negative along ripening.

The K_{225} index for the two olive oils is shown in Figure 6. This value decreases along ripening. The value was higher for Arbequina olive oils in the 2011 crop season . In 2012 the value higher was for Tosca 07® olive oils. The K_{225} tendency was related in all the cases to the phenol content.

CONCLUSIONS

Tosca 07® is a very interesting olive variety for high density orchards. Because of their early ripening, they would be suitable for early harvesting, and this could be interesting to avoid the cold temperatures, frost, etc. At early harvesting, Tosca 07® olive oils have an interesting composition in comparison with Arbequina olive oils, especially for the antioxidant compounds (high α- tocopherol and pigments content) and other nutritional compounds, such as some essential fatty acids.

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Figure and table legends

Figure 1- Temperature registration in the orchard during 2001 and 2012 crops.

Figure 2- Rainfall registration in the orchard during 2001 and 2012 crops.

Figure 3-Total phenol content of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Figure 4-. α-tocopherol content of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Figure 5- Oxidative stability of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Figure 6-. Bitterness index of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Table 1. Morphological and physicochemical parameters of olives and olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Table 2. Fatty acid composition of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Table 3. Individual phenols of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Table 4. Pigments and colour coordinates of olive oils from Tosca 07® and Arbequina during 2011 and 2012 crops.

Table 1.

Parameters	Year 2011						Year 2012					
	Tosca olive oil			Arbequina olive oil			Tosca olive oil			Arbequina olive oil		
	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov
Ripeness index	$3.9\pm0.0^{A;X;F}$	$4.3\pm0.1^{AB;X;F}$	$4.4\pm0.1^{B;X;F}$	$1.9\pm0.0^{A;X;E}$	$2.0\pm0.1^{A;X;E}$	$2.7\pm0.0^{B;X;E}$	$3.3\pm0.0^{A;X;F}$	$3.7\pm0.1^{A;X;F}$	$3.8\pm0.0^{A;X;F}$	$1.4\pm0.0^{A;X;E}$	$2.2\pm0.1^{B;X;E}$	$2.4\pm0.1^{B;X;E}$
Olive weight (g)	$1.9\pm0.1^{A;Y;F}$	$2.1\pm0.1^{B;X;F}$	$2.3\pm0.1^{C;Y;F}$	$1.5\pm0.1^{A;X;E}$	$1.6\pm0.0^{A;X;E}$	$1.8\pm0.0^{B;X;E}$	$1.7\pm0.1^{A;X;F}$	$2.0\pm0.1^{B;X;F}$	$2.1\pm0.0^{B;X;F}$	$1.3\pm0.1^{A;X;E}$	$1.6\pm0.1^{B;X;E}$	$1.9\pm0.1^{C;X;E}$
Polar diameter (mm)	$16.0\pm0.9^{A;X;F}$	$16.5\pm1.0^{B;X;F}$	$17.3\pm1.1^{C;Y;F}$	$14.4\pm1.1^{A;X;E}$	$14.4\pm1.2^{A;X;E}$	$14.2\pm0.9^{A;X;E}$	$16.0\pm1.0^{A;X;F}$	$16.4\pm1.4^{A;X;F}$	$16.0\pm1.1^{A;X;F}$	$14.6\pm0.9^{A;X;E}$	$15.3\pm0.9^{B;Y;E}$	$15.1\pm0.9^{B;Y;E}$
Equatorial diameter (mm)	$13.8\pm0.6^{A;X;F}$	$14.3\pm0.8^{B;X;F}$	$14.8\pm0.7^{C;Y;F}$	$13.2\pm0.9^{A;Y;E}$	$13.4\pm1.0^{A;X;E}$	$13.2\pm0.8^{A;X;E}$	$13.9\pm0.8^{A;X;F}$	$14.4\pm0.9^{B;X;F}$	$14.2\pm0.7^{AB;X;F}$	$12.7\pm0.8^{A;X;E}$	$13.4\pm0.8^{B;X;E}$	$13.5\pm0.9^{B;X;E}$
Oil content/dry matter in olive paste (%)	$44.6\pm0.6^{A;X;E}$	$49.2\pm0.2^{B;X;E}$	$49.5\pm0.7^{B;X;E}$	$52.7\pm1.4^{A;X;F}$	$54.3\pm0.1^{A;X;F}$	$52.3\pm1.8^{A;X;E}$	$45.0\pm0.1^{A;X;E}$	$53.7\pm0.3^{C;Y;E}$	$48.1\pm0.2^{B;X;E}$	$46.6\pm1.4^{A;X;E}$	$52.5\pm0.8^{B;X;E}$	$52.4\pm0.6^{B;X;F}$
Acidity (% oleic acid)	$0.14\pm0.01^{A;X;F}$	$0.17\pm0.01^{B;X;F}$	$0.21\pm0.01^{C;X;F}$	$0.11\pm0.01^{A;X;E}$	$0.12\pm0.01^{A;X;E}$	$0.14\pm0.00^{B;X;E}$	$0.14\pm0.01^{A;X;F}$	$0.17\pm0.01^{B;X;F}$	$0.21\pm0.01^{C;X;F}$	$0.11\pm0.01^{A;X;E}$	$0.12\pm0.01^{A;X;E}$	$0.14\pm0.00^{B;X;E}$
Peroxide value (meq O_2 active/Kg oil)	$13.3\pm0.1^{A;X;F}$	$16.6\pm0.0^{C;Y;F}$	$15.3\pm0.0^{B;X;F}$	$5.3\pm0.1^{A;Y;E}$	$7.3\pm0.0^{C;Y;E}$	$6.7\pm0.0^{B;Y;E}$	$15.3\pm0.1^{B;Y;F}$	$15.0\pm0.1^{A;X;F}$	$15.6\pm0.1^{C;Y;F}$	$3.3\pm0.0^{A;X;E}$	$3.3\pm0.0^{A;X;E}$	$3.3\pm0.0^{A;X;E}$
K_{232} (abs a 232 nm)	$2.0\pm0.0^{A;Y;F}$	$2.2\pm0.0^{B;Y;F}$	$2.2\pm0.0^{B;Y;F}$	$1.4\pm0.0^{A;Y;E}$	$1.6\pm0.0^{C;Y;E}$	$1.6\pm0.0^{B;Y;E}$	$1.8\pm0.0^{A;X;F}$	$1.8\pm0.0^{A;X;F}$	$1.8\pm0.0^{A;X;F}$	$1.3\pm0.0^{B;X;E}$	$1.3\pm0.0^{B;X;E}$	$1.2\pm0.0^{A;X;E}$
K_{270} (abs a 270 nm)	$0.13\pm0.00^{C;Y;F}$	$0.12\pm0.00^{B;X;F}$	$0.11\pm0.00^{A;X;E}$	$0.10\pm0.00^{A;X;E}$	$0.11\pm0.00^{A;Y;E}$	$0.09\pm0.00^{A;X;E}$	$0.11\pm0.00^{A;X;F}$	$0.12\pm0.00^{B;X;F}$	$0.11\pm0.00^{A;X;F}$	$0.10\pm0.00^{C;X;E}$	$0.09\pm0.00^{B;X;E}$	$0.08\pm0.00^{A;X;E}$

Values reported are mean values and standard deviations of three replicates.

[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.

[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.

[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Table 2.

Parameters	Year 2011						Year 2012					
	Tosca olive oil			Arbequina olive oil			Tosca olive oil			Arbequina olive oil		
	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov
Palmitic acid (%)	15.6±0.0$^{C;Y;E}$	15.4±0.0$^{B;X;E}$	15.2±0.0$^{A;X;E}$	16.7±0.0$^{B;Y;F}$	16.3±0.0$^{A;Y;F}$	16.2±0.1$^{A;Y;F}$	15.5±0.0$^{B;X;E}$	15.5±0.0$^{B;X;F}$	15.3±0.0$^{A;Y;E}$	15.4±0.2$^{A;X;E}$	15.3±0.0$^{A;X;E}$	15.2±0.2$^{A;X;E}$
Palmitoleic acid (%)	1.1±0.1$^{A;X;E}$	1.1±0.1$^{A;X;E}$	1.0±0.0$^{A;X;E}$	1.8±0.0$^{A;Y;F}$	1.8±0.1$^{A;X;F}$	1.7±0.0$^{A;Y;F}$	0.95±0.00$^{A;X;E}$	1.00±0.01$^{AB;X;E}$	1.00±0.02$^{B;X;E}$	1.5±0.0$^{B;X;F}$	1.6±0.0$^{B;X;F}$	1.5±0.0$^{A;X;F}$
Margaric acid (%)	0.04±0.00$^{A;X;E}$	0.04±0.00$^{A;X;E}$	0.04±0.00$^{A;X;E}$	0.13±0.00$^{A;X;F}$	0.13±0.00$^{A;X;F}$	0.13±0.00$^{A;X;F}$	0.05±0.01$^{A;X;E}$	0.04±0.01$^{A;X;E}$	0.05±0.01$^{A;X;E}$	0.14±0.01$^{A;X;F}$	0.13±0.00$^{A;X;F}$	0.14±0.00$^{A;X;F}$
Margaroleic acid (%)	0.07±0.00$^{A;X;E}$	0.07±0.00$^{A;X;E}$	0.08±0.01$^{A;X;E}$	0.26±0.01$^{A;X;F}$	0.25±0.00$^{A;X;F}$	0.25±0.00$^{A;X;F}$	0.08±0.00$^{A;X;E}$	0.09±0.00$^{B;Y;E}$	0.09±0.00$^{B;X;E}$	0.27±0.00$^{A;X;F}$	0.27±0.01$^{A;X;F}$	0.26±0.01$^{A;X;F}$
Estearic acid (%)	2.0±0.0$^{A;X;F}$	2.2±0.0$^{B;Y;F}$	2.4±0.0$^{C;Y;F}$	1.9±0.0$^{B;X;E}$	1.9±0.0$^{B;X;E}$	1.9±0.0$^{A;X;E}$	2.1±0.0$^{A;X;F}$	2.1±0.0$^{A;X;F}$	2.2±0.0$^{A;X;F}$	2.0±0.0$^{A;X;E}$	2.0±0.0$^{A;X;E}$	2.0±0.0$^{A;Y;E}$
Oleic acid (%)	64.7±0.1$^{C;X;E}$	64.2±0.1$^{B;X;E}$	63.6±0.1$^{A;X;E}$	66.5±0.0$^{A;X;F}$	66.9±0.1$^{B;X;F}$	67.0±0.1$^{B;X;F}$	66.5±0.0$^{C;Y;E}$	65.7±0.1$^{B;Y;E}$	65.4±0.0$^{A;Y;E}$	69.5±0.1$^{A;Y;F}$	69.1±0.1$^{A;Y;F}$	69.3±0.2$^{A;Y;F}$
Linoleic acid (%)	14.8±0.0$^{A;Y;F}$	15.4±0.0$^{B;Y;F}$	16.1±0.0$^{C;Y;F}$	11.5±0.0$^{A;Y;E}$	11.5±0.0$^{A;Y;E}$	11.7±0.2$^{A;Y;E}$	13.1±0.0$^{A;X;F}$	14.0±0.1$^{B;X;F}$	14.4±0.0$^{C;X;F}$	9.8±0.0$^{A;X;E}$	10.3±0.0$^{B;X;E}$	10.3±0.0$^{B;X;E}$
Linolenic acid (%)	1.00±0.00$^{B;X;F}$	1.00±0.01$^{B;X;F}$	0.98±0.01$^{A;X;F}$	0.57±0.01$^{A;X;E}$	0.56±0.02$^{A;X;E}$	0.55±0.00$^{A;X;E}$	1.00±0.00$^{B;X;F}$	0.98±0.01$^{A;X;F}$	0.95±0.00$^{A;X;F}$	0.63±0.00$^{B;Y;E}$	0.61±0.00$^{A;Y;E}$	0.62±0.01$^{B;Y;E}$
Arachidic acid (%)	0.35±0.01$^{A;X;E}$	0.35±0.01$^{A;X;E}$	0.37±0.00$^{A;X;E}$	0.40±0.01$^{A;X;E}$	0.40±0.00$^{A;X;F}$	0.39±0.00$^{A;X;F}$	0.38±0.00$^{B;X;E}$	0.38±0.00$^{B;Y;E}$	0.36±0.01$^{A;X;E}$	0.40±0.00$^{A;X;F}$	0.39±0.01$^{A;X;E}$	0.42±0.01$^{A;X;F}$
Gadoleic acid (%)	0.24±0.01$^{A;X;E}$	0.25±0.00$^{A;X;E}$	0.24±0.01$^{A;X;E}$	0.26±0.01$^{A;X;E}$	0.27±0.01$^{A;X;E}$	0.26±0.00$^{A;X;E}$	0.31±0.02$^{A;Y;E}$	0.30±0.01$^{A;Y;E}$	0.29±0.01$^{A;Y;E}$	0.31±0.00$^{A;Y;E}$	0.31±0.02$^{A;X;E}$	0.32±0.00$^{A;Y;F}$
SFA (%)	18.1±0.0$^{B;X;E}$	18.0±0.0$^{A;X;E}$	18.0±0.0$^{A;Y;E}$	19.1±0.0$^{B;Y;F}$	18.7±0.0$^{A;Y;F}$	18.6±0.1$^{A;Y;F}$	18.0±0.0$^{B;X;E}$	18.0±0.0$^{B;X;F}$	17.9±0.0$^{A;X;E}$	18.0±0.1$^{A;X;E}$	17.8±0.0$^{A;X;E}$	17.7±0.2$^{A;X;E}$
MUFAS (%)	66.1±0.0$^{C;X;E}$	65.6±0.0$^{B;X;E}$	65.0±0.0$^{A;X;E}$	68.8±0.0$^{A;X;F}$	69.3±0.0$^{B;X;F}$	69.3±0.1$^{B;X;F}$	67.9±0.0$^{C;Y;E}$	67.1±0.1$^{B;Y;E}$	66.8±0.0$^{A;Y;E}$	71.6±0.1$^{A;Y;F}$	71.2±0.1$^{A;Y;F}$	71.3±0.2$^{A;Y;F}$
PUFAS (%)	15.9±0.0$^{A;Y;F}$	16.4±0.0$^{B;Y;F}$	17.1±0.0$^{C;Y;F}$	12.1±0.0$^{A;Y;E}$	12.0±0.0$^{A;Y;E}$	12.2±0.2$^{A;Y;E}$	14.1±0.0$^{A;X;F}$	14.9±0.1$^{B;X;F}$	15.3±0.0$^{C;X;F}$	10.4±0.0$^{A;X;E}$	10.9±0.0$^{B;X;E}$	10.9±0.0$^{B;X;E}$
MUFAS/PUFAS	4.2±0.0$^{C;X;E}$	4.0±0.0$^{B;X;E}$	3.8±0.0$^{A;X;E}$	5.7±0.0$^{A;X;F}$	5.8±0.0$^{A;X;F}$	5.7±0.1$^{A;X;F}$	4.8±0.0$^{C;Y;E}$	4.5±0.0$^{B;Y;E}$	4.4±0.0$^{A;Y;E}$	6.9±0.0$^{B;Y;F}$	6.5±0.0$^{A;Y;F}$	6.5±0.0$^{A;Y;F}$

Values reported are mean values and standard deviations of three replicates.
SFA, saturated fatty acids; MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids.

$^{A-C}$For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.
$^{X-Y}$For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.
$^{E-F}$For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Table 3.

Parameters	Year 2011						Year 2012					
	Tosca olive oil			Arbequina olive oil			Tosca olive oil			Arbequina olive oil		
	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov
Hydroxytyrosol (mg/Kg)	$0.44\pm0.00^{C;X;F}$	$0.34\pm0.01^{B;X;F}$	$0.26\pm0.01^{A;X;E}$	$0.36\pm0.00^{B;X;E}$	$0.27\pm0.00^{A;X;E}$	$0.26\pm0.00^{A;X;E}$	$0.51\pm0.00^{A;Y;E}$	$0.51\pm0.00^{A;Y;E}$	$0.51\pm0.00^{A;Y;E}$	$0.53\pm0.00^{C;Y;F}$	$0.52\pm0.00^{B;Y;F}$	$0.51\pm0.00^{A;Y;F}$
Tyrosol (mg/Kg)	$3.2\pm0.0^{A;Y;F}$	$3.7\pm0.0^{C;Y;F}$	$3.5\pm0.0^{B;X;F}$	$0.91\pm0.05^{B;X;E}$	$0.84\pm0.04^{AB;X;E}$	$0.82\pm0.02^{A;X;E}$	$2.4\pm0.0^{A;X;F}$	$3.1\pm0.1^{B;X;F}$	$3.6\pm0.0^{C;Y;F}$	$0.94\pm0.02^{A;X;E}$	$0.99\pm0.03^{B;Y;E}$	$1.1\pm0.0^{C;Y;E}$
Vanillic acid (mg/Kg)	$0.30\pm0.01^{A;X;E}$	$0.37\pm0.01^{C;X;E}$	$0.32\pm0.00^{B;X;E}$	$0.50\pm0.02^{C;X;F}$	$0.45\pm0.01^{B;X;F}$	$0.34\pm0.01^{A;X;E}$	$0.42\pm0.01^{A;Y;E}$	$0.48\pm0.01^{B;Y;E}$	$0.48\pm0.01^{B;Y;E}$	$0.78\pm0.01^{C;Y;F}$	$0.56\pm0.02^{A;Y;F}$	$0.75\pm0.01^{B;Y;F}$
Vanillin (mg/Kg)	$1.4\pm0.0^{B;Y;E}$	$1.4\pm0.0^{B;Y;E}$	$1.3\pm0.0^{A;X;E}$	$1.5\pm0.0^{B;X;F}$	$1.5\pm0.0^{B;X;F}$	$1.4\pm0.0^{A;X;F}$	$1.3\pm0.0^{A;X;E}$	$1.4\pm0.0^{C;X;E}$	$1.3\pm0.0^{B;Y;E}$	$1.6\pm0.0^{B;Y;F}$	$1.5\pm0.0^{A;Y;F}$	$1.5\pm0.0^{A;Y;F}$
Coumaric acid (mg/Kg)	$0.53\pm0.01^{B;X;E}$	$0.48\pm0.00^{A;X;E}$	$0.48\pm0.01^{A;X;E}$	$1.6\pm0.0^{C;Y;F}$	$1.1\pm0.0^{A;Y;F}$	$1.3\pm0.0^{B;Y;F}$	$0.68\pm0.01^{A;Y;E}$	$0.72\pm0.01^{B;Y;E}$	$0.77\pm0.01^{C;Y;E}$	$0.78\pm0.01^{B;X;F}$	$0.75\pm0.00^{A;X;F}$	$0.78\pm0.00^{B;X;E}$
3.4-DHPEA-AC (mg/Kg)	$24.7\pm0.2^{C;X;E}$	$21.6\pm0.3^{B;X;E}$	$7.6\pm0.1^{A;X;E}$	$49.0\pm0.7^{B;Y;F}$	$57.9\pm0.1^{C;Y;F}$	$34.9\pm0.3^{A;Y;F}$	$45.2\pm0.5^{A;Y;F}$	$75.5\pm0.6^{C;Y;F}$	$46.6\pm0.4^{B;Y;F}$	$27.2\pm0.4^{A;X;E}$	$30.1\pm0.6^{B;X;E}$	$31.5\pm0.4^{C;X;E}$
3.4-DHPEA-EDA (mg/Kg)	$22.1\pm0.3^{C;X;E}$	$18.5\pm0.1^{B;X;E}$	$7.6\pm0.1^{A;X;E}$	$239.4\pm0.4^{C;Y;F}$	$166.5\pm0.6^{B;Y;F}$	$120.3\pm0.5^{A;Y;F}$	$60.1\pm0.2^{B;Y;E}$	$64.3\pm0.1^{C;Y;E}$	$38.3\pm0.1^{A;Y;E}$	$83.3\pm0.1^{B;X;F}$	$81.9\pm0.2^{A;X;F}$	$83.0\pm0.1^{B;X;F}$
p-HPEA-EDA (mg/Kg)	$14.9\pm0.3^{C;X;E}$	$14.0\pm0.1^{B;X;E}$	$12.1\pm0.0^{A;X;E}$	$35.7\pm0.1^{C;Y;F}$	$32.5\pm0.1^{B;Y;F}$	$18.0\pm0.1^{A;Y;F}$	$36.2\pm0.1^{C;Y;F}$	$34.1\pm0.1^{B;Y;F}$	$24.8\pm0.2^{A;Y;F}$	$21.6\pm0.3^{C;X;E}$	$17.6\pm0.0^{B;X;E}$	$16.0\pm0.2^{A;X;E}$
Lignans (mg/Kg)	$39.0\pm0.3^{A;Y;F}$	$40.4\pm0.2^{B;X;F}$	$41.5\pm0.2^{C;X;F}$	$29.9\pm0.2^{A;X;E}$	$29.6\pm0.1^{A;X;E}$	$33.2\pm0.3^{B;X;E}$	$34.4\pm0.1^{A;X;E}$	$44.3\pm0.1^{C;Y;F}$	$43.1\pm0.1^{B;Y;F}$	$35.5\pm0.1^{C;Y;F}$	$32.6\pm0.1^{A;Y;E}$	$34.9\pm0.1^{B;Y;E}$
3.4-DHPEA-EA (mg/Kg)	$22.9\pm1.3^{A;Y;F}$	$28.8\pm0.8^{B;Y;F}$	$36.6\pm0.5^{C;Y;F}$	$15.7\pm0.4^{C;X;E}$	$13.5\pm0.3^{B;X;E}$	$10.9\pm0.5^{A;X;E}$	$19.0\pm0.3^{A;X;E}$	$24.5\pm0.4^{B;X;F}$	$30.1\pm0.5^{C;X;F}$	$24.0\pm0.3^{B;Y;F}$	$21.2\pm0.3^{A;Y;E}$	$21.3\pm0.2^{A;Y;E}$
Luteolin (mg/Kg)	$8.2\pm0.0^{A;X;F}$	$8.9\pm0.0^{C;X;F}$	$8.6\pm0.0^{B;X;F}$	$5.5\pm0.0^{B;Y;E}$	$4.6\pm0.0^{A;Y;E}$	$6.0\pm0.0^{C;Y;E}$	$8.1\pm0.0^{A;X;F}$	$9.6\pm0.0^{C;Y;F}$	$9.4\pm0.0^{B;Y;F}$	$3.8\pm0.0^{B;X;E}$	$3.6\pm0.0^{A;X;E}$	$4.9\pm0.0^{C;X;E}$
Apigenin (mg/Kg)	$4.4\pm0.0^{A;X;F}$	$4.6\pm0.0^{B;X;F}$	$4.6\pm0.0^{B;X;F}$	$2.5\pm0.0^{B;Y;E}$	$2.4\pm0.0^{A;Y;E}$	$2.8\pm0.0^{C;Y;E}$	$4.4\pm0.0^{A;X;F}$	$5.5\pm0.0^{B;Y;F}$	$5.5\pm0.0^{B;Y;F}$	$2.0\pm0.0^{B;X;E}$	$1.9\pm0.0^{A;X;E}$	$2.5\pm0.0^{C;X;E}$

Values reported are mean values and standard deviations of three replicates.
3.4-DHPEA-AC, 4-(acetoxyethyl)-1.2-dihydroxybenzene; 3.4-DHPEA-EDA, dialdehydric form of elenolic acid linked to hydroxytyrosol; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3.4-DHPEA-EA, oleuropein aglycone.

$^{A-C}$For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.
$^{X-Y}$For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.
$^{E-F}$For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Table 4.

Parameters	Year 2011						Year 2012					
	Tosca olive oil			Arbequina olive oil			Tosca olive oil			Arbequina olive oil		
	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov	19 Oct	26 Oct	02 Nov
Pigments												
Chlorophyll (mg/Kg)	$11.2\pm0.0^{C;X;F}$	$7.6\pm0.0^{B;Y;F}$	$3.4\pm0.0^{A;X;E}$	$8.0\pm0.0^{C;X;E}$	$6.7\pm0.1^{B;Y;E}$	$4.1\pm0.0^{A;X;F}$	$12.0\pm0.0^{C;Y;F}$	$6.6\pm0.1^{B;X;F}$	$3.4\pm0.0^{A;X;E}$	$9.9\pm0.1^{C;Y;E}$	$5.6\pm0.1^{B;X;E}$	$4.2\pm0.1^{A;X;F}$
Carotenoids (mg/Kg)	$9.1\pm0.0^{C;Y;F}$	$7.0\pm0.0^{B;Y;F}$	$4.0\pm0.0^{A;Y;F}$	$6.8\pm0.0^{C;Y;E}$	$5.8\pm0.0^{B;Y;E}$	$3.6\pm0.0^{A;Y;E}$	$7.6\pm0.1^{C;X;F}$	$4.8\pm0.0^{B;X;F}$	$3.8\pm0.0^{A;X;F}$	$6.6\pm0.1^{C;X;E}$	$4.2\pm0.0^{B;X;E}$	$3.5\pm0.0^{A;X;E}$
Color coordinates												
L*	$86.0\pm0.0^{A;X;E}$	$88.0\pm0.1^{B;X;E}$	$92.5\pm0.1^{C;X;E}$	$88.3\pm0.3^{A;X;F}$	$89.1\pm0.4^{B;X;F}$	$92.6\pm0.2^{C;X;E}$	$94.4\pm0.0^{A;Y;E}$	$98.4\pm0.5^{B;Y;E}$	$101.5\pm0.2^{C;Y;F}$	$96.1\pm0.3^{A;Y;F}$	$99.7\pm0.5^{B;Y;F}$	$100.2\pm0.4^{B;Y;E}$
a*	$-0.47\pm0.02^{C;Y;F}$	$-1.4\pm0.0^{B;Y;F}$	$-4.3\pm0.0^{A;Y;F}$	$-2.0\pm0.1^{C;Y;E}$	$-2.4\pm0.1^{B;Y;E}$	$-4.4\pm0.0^{A;Y;E}$	$-15.8\pm0.1^{C;X;E}$	$-16.2\pm0.1^{B;X;F}$	$-17.7\pm0.0^{A;X;F}$	$-15.2\pm0.0^{C;X;F}$	$-16.6\pm0.1^{B;X;E}$	$-18.9\pm0.2^{A;X;E}$
b*	$115.9\pm0.2^{C;Y;F}$	$104.0\pm0.1^{B;Y;F}$	$77.6\pm0.1^{A;X;F}$	$103.2\pm0.7^{C;X;E}$	$91.4\pm0.8^{B;Y;E}$	$73.5\pm0.2^{A;X;E}$	$114.2\pm0.5^{C;X;F}$	$96.0\pm0.4^{B;X;F}$	$84.9\pm0.4^{A;Y;F}$	$110.7\pm0.4^{C;Y;E}$	$90.1\pm0.6^{B;X;E}$	$81.9\pm0.6^{A;Y;E}$

Values reported are mean values and standard deviations of three replicates.
[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p\leq0.05$) among picking dates.
[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p\leq0.05$) between years.
[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p\leq0.05$) between varieties.

Figure 1.

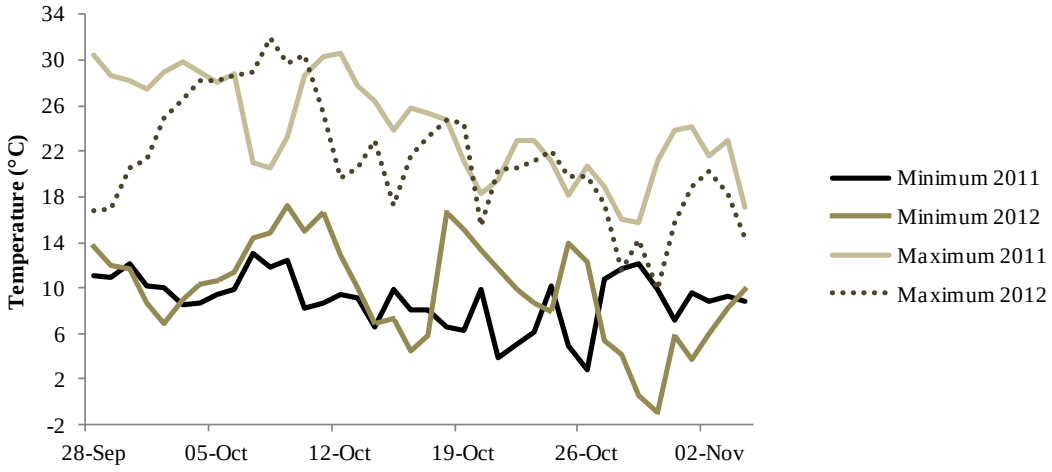


Figure 2.

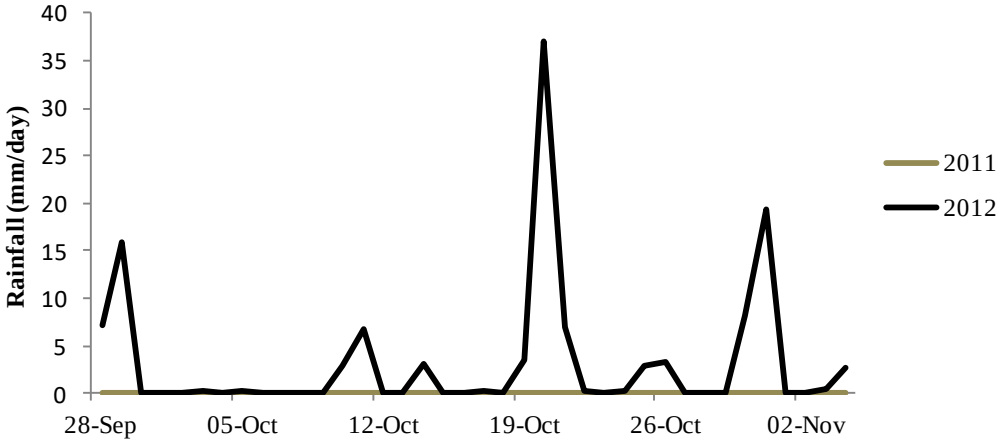
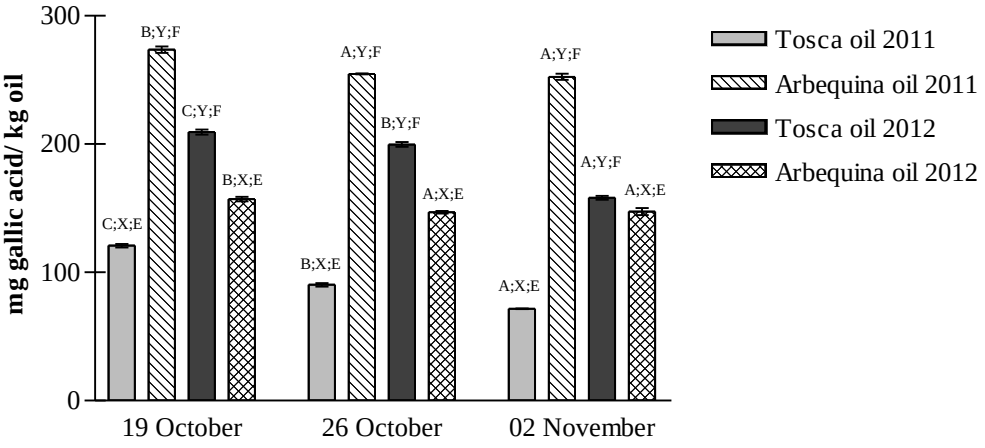


Figure 3.

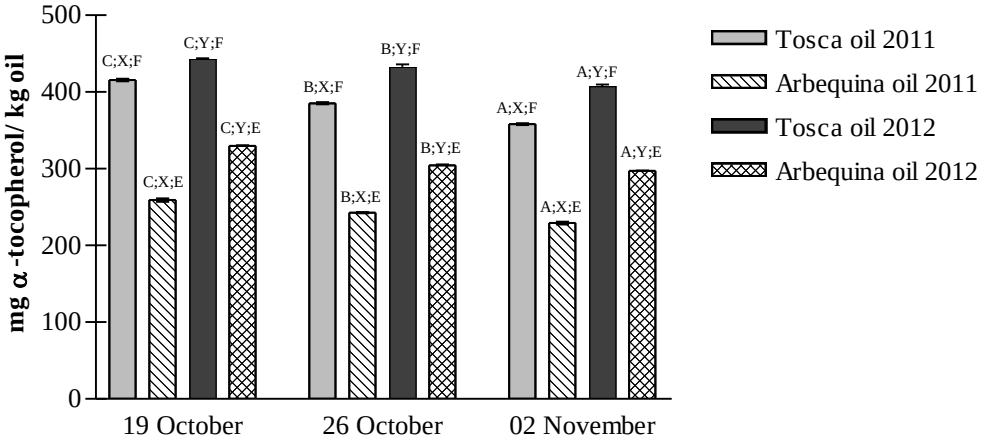


Values reported are mean values and standard deviations of three replicates.
[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.

[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.

[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Figure 4.

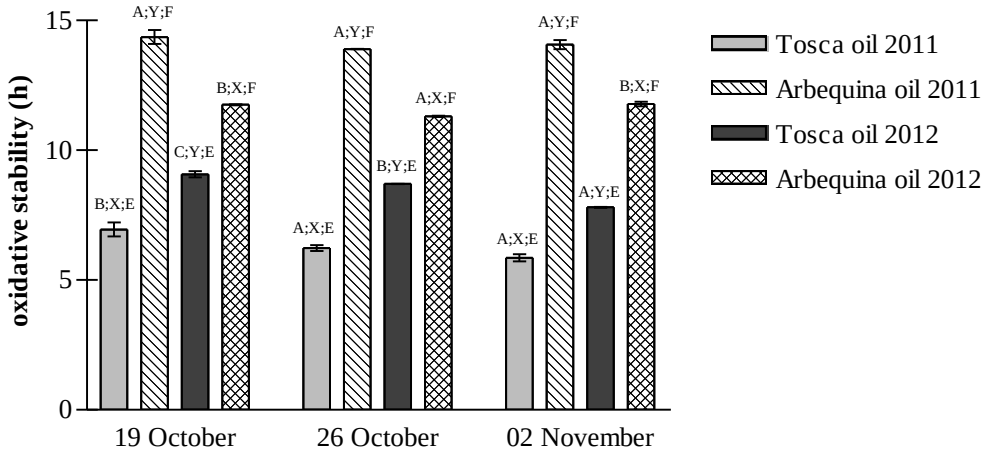


Values reported are mean values and standard deviations of three replicates.
[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.

[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.

[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Figure 5.

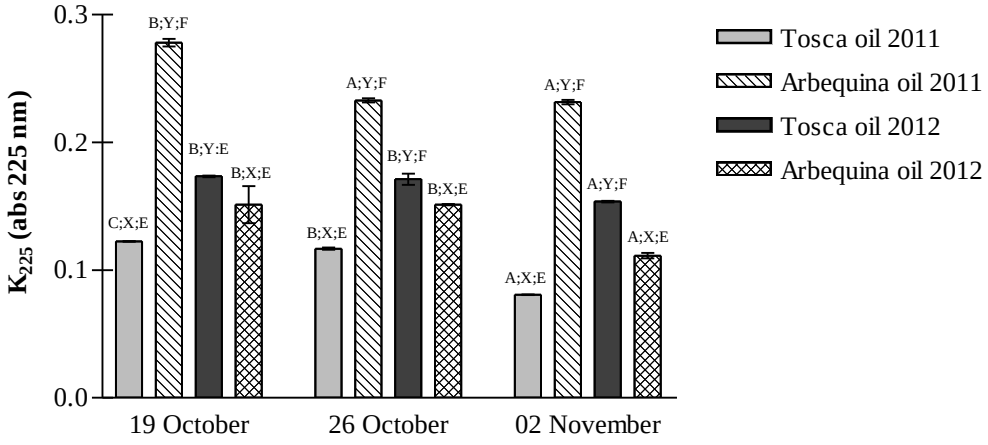


Values reported are mean values and standard deviations of three replicates.
[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.

[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.

[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.

Figure 6.



Values reported are mean values and standard deviations of three replicates.
[A-C] For each parameter, different letters for the same year and variety indicate statistically significant differences ($p \leq 0.05$) among picking dates.

[X-Y] For each parameter, different letters for the same variety and picking date indicate statistically significant differences ($p \leq 0.05$) between years.

[E-F] For each parameter, different letters for the same picking date and year indicate statistically significant differences ($p \leq 0.05$) between varieties.